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# Longitudinal analysis of levels of immunoglobulins against BK virus capsid proteins in kidney transplant recipients

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## Longitudinal Analysis of Levels of Immunoglobulins against BK Virus Capsid Proteins in Kidney Transplant Recipients<sup>†</sup>

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**This study sought to evaluate serology and PCR as tools for measuring BK virus (BKV) replication. Levels of immunoglobulin G (IgG), IgM, and IgA against BKV capsids were measured at five time points for 535 serial samples from 107 patients by using a virus-like particle-based enzyme-linked immunosorbent assay. Viral DNA in urine and plasma samples was quantitated. The seroconversion rate was 87.5% (14/16); 78.6% (11/14) and 14.3% (2/14) of patients who seroconverted developed viruria and viremia, respectively. Transient seroreversion was observed in 18.7% of patients at  $17.4 \pm 11.9$  weeks posttransplant and was not attributable to loss of antigenic stimulation, changes in immunosuppression, or antiviral treatment. Titers for anti-BK IgG, IgA, and IgM were higher in patients with BKV replication than in those without BKV replication. A rise in the optical density (OD) of anti-BK IgA (0.19), IgM (0.04), or IgG (0.38) had a sensitivity of 76.6 to 88.0% and a specificity of 71.7 to 76.1% for detection of viruria. An anti-BK IgG- and IgA-positive phenotype at week 1 was less frequent in patients who subsequently developed viremia (14.3%) than in those who subsequently developed viruria (42.2%) ( $P = 0.04$ ). Anti-BK IgG OD at week 1 showed a weak negative correlation with peak urine viral load ( $r = -0.25$ ;  $P = 0.05$ ). In summary, serial measurements of anti-BKV immunoglobulin class (i) detect onset of viral replication, (ii) document episodes of seroreversion, and (iii) can potentially provide prognostic information.**

BK virus (BKV) belongs to the genus *Polyomaviridae* and is now a well-recognized pathogen in kidney transplant recipients. Its virions are 45 nm in diameter and have a 5-kb double-stranded, circular, supercoiled DNA, which is encased in a protein capsid (11). Primary infection is believed to occur early in life via the respiratory route. This is followed by viral latency in the urogenital tract. BKV reactivation with urinary excretion of virus occurs in 10 to 60% of kidney transplant recipients. Viremia develops in 5 to 25% patients, and approximately 1 to 10% have biopsy-documented viral nephropathy (BKVN) (14–16, 27, 34, 35, 39–41, 43, 45, 51). BKVN has also been described to occur in the native kidneys of liver, heart, and bone marrow transplant recipients and in patients with congenital immunodeficiency or AIDS (10, 44, 45, 51). Diagnosis of BKVN is primarily based on histological examination, although persistent viremia has been used as a surrogate marker for this complication (36). Initial clinical series of BKVN were characterized by a graft loss that exceeded 50%. In recent years, intensive monitoring techniques have been used to facilitate early diagnosis and prevent irreversible graft injury leading to graft loss.

Much remains to be learned about the humoral immune response to polyomavirus infection in humans. Classic publi-

cations were based primarily on hemagglutination inhibition assays (12, 13, 17, 18, 19, 28, 31, 37, 47). Immunoglobulin classes were usually not measured, and the patients studied were not well characterized from a clinical perspective. Several recent studies have used enzyme-linked immunosorbent assay (ELISA) technology and have shown marked elevations in anti-BKV antibodies in patients with active viral replication (4, 22, 23, 31, 42). It has been difficult to show definitively whether this intense humoral immune response actually contributes to resolution of viral nephropathy. Available data suggest that early infection can be frequently derived from the donor kidney (4, 5). The use of serial antibody testing as a tool to screen for BKV infection remains to be evaluated formally. There are also few data on the clinical utility of measuring immunoglobulin isotypes with respect to diagnosis and prognosis. To address these issues, we have measured levels of immunoglobulin G (IgG), IgA, and IgM antibodies against BKV virus-like particles (VLPs) in a well-characterized set of 535 samples derived from 107 kidney transplant recipients.

### MATERIALS AND METHODS

This study is based on preexisting, deidentified human specimens and associated clinical data obtained from the Washington University Kidney Translational Research Core (KTRC). Institutional Review Board approval was obtained at Washington University under the original protocol “A Randomized Prospective Controlled Clinical and Pharmacoeconomic Study of Cyclosporine versus Tacrolimus in Adult Renal Transplant Recipients,” Washington University School of Medicine, Human Studies Committee no. 00-0951, with continuation under the title “Polyomavirus and Mentoring in Renal Transplantation.” Detailed clinical features and immunosuppression protocols for these patients have previously been published (1, 5, 6). Briefly, these patients were enrolled in a prospective open-label trial and randomized to receive tacrolimus or cyclospo-

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rine in a 2:1 block design. Exclusion criteria included patients who were pregnant, nursing, or seropositive for other viral infections (human immunodeficiency virus, human T-cell leukemia virus type 1, or hepatitis virus). Patients with histories of rabbit anti-T-cell polyclonal agents were also not included. The age ranges were  $44 \pm 13$  years for tacrolimus-treated patients and  $46 \pm 13$  years for cyclosporine-treated patients. Males constituted 64% and 61% of these two treatment arms, respectively. Overall, 64 patients in this study developed viruria on at least one occasion, of which 14 also became viremic. As defined by positive test results for viral DNA with two consecutive samples, 48 patients had persistent viruria, while only 2 had persistent viremia. At the 1-year follow-up, 40 patients remained viruric, but none was viremic.

Recipients were prospectively monitored for BK viruria, viremia, and nephropathy. Identification of viremia triggered discontinuation of the antimetabolite component of the immunosuppressive regimen. This maneuver successfully prevented the development of nephropathy in this cohort, although no protocol biopsies were performed to rule out subclinical nephropathy.

A total of 535 serial plasma and urine samples were retrieved from 107 patients, of whom 50 had viruria, 14 had both viruria and viremia, and 43 were negative for BKV DNA by PCR. No patient had viremia in the absence of viruria. These were all essentially "samples of convenience" that had previously been collected at 1, 4, and 12 weeks; 6 months; and 1 year posttransplant with the aforementioned protocol. BKV DNA was measured by a published quantitative PCR assay that targets the large T-antigen gene and has a lower limit of sensitivity equivalent to 4,000 viral copies per ml (1). BK viruria and viremia were defined as the presence of viral DNA as determined by PCR in urine and plasma samples, respectively. Detection of viral DNA in either fluid was regarded as evidence of viral replication.

For this study, BKV-specific antibodies in plasma samples were measured using a VLP-based ELISA (42). This assay does not show cross-reactivity with anti-JC virus antibodies. Briefly, the ELISA protocol consisted of coating microtiter plates with purified VLP protein (0.2  $\mu$ g/well), followed by treatment with a blocking solution. Diluted serum samples (1:100 for IgA and IgM and 1:200 for IgG), in duplicate, were allowed to react on the antigen-coated plates, and antigen-bound immunoglobulin was detected with peroxidase-conjugated antibodies to human IgG, IgA, or IgM (Southern BioTech, Birmingham, AL). IgA and IgM measurements were performed after an initial blocking step with 15% goat anti-human IgG (Sigma-Aldrich, St. Louis, MO). Color development was initiated by addition of substrate, and optical density (OD) was measured at 405 nm with an automated microtiter plate reader. Positive and negative control sera, sensitivity controls, and reproducibility controls were included in each run. Runs where replicate serum values fell outside the expected coefficient of variation (7.9%) were repeated. Results are recorded both as OD values and as categorical variables (seropositive or seronegative) based on cutoff points determined by assaying known seronegative subjects.

The OD cutoff points used to distinguish seronegative and seropositive subjects for the VLP batch used in this study were 0.075, 0.150, and 0.080 for anti-BK IgG, IgA, and IgM antibodies, respectively. For every patient, change in anti-BK IgG ( $\Delta$  anti-BK IgG), IgA, or IgM was defined as the difference between the peak OD and the baseline OD ( $\Delta$  OD) for the immunoglobulin class of interest. Patients who were seronegative for BKV at week 1 and subsequently became seropositive were considered to represent primary infection, although we could not definitively rule out a prior exposure with subsequent seroreversion or an antibody level below the detection limit of the assay. Reactivation infection was defined as the appearance of anti-BK IgA or IgM antibodies in patients who had only an anti-BK IgG antibody detected at week 1. Patients who had anti-BK IgA or IgM detectable at week 1 could not be definitively categorized as having either primary or reactivation infection, since time zero samples were not analyzed. However, given the high seroprevalence for BKV in healthy donors, it is likely that the majority of these were reactivation infections.

For statistical analysis, basic demographics were described using measures of central tendency (means and medians) and spread (standard deviation and range) for continuous data and frequencies and percentages for categorical data. Comparisons between groups were performed using rank sum and exact chi-square tests. Correlations between viral load and serologic parameters were obtained using Spearman correlation coefficients. To identify potential cutoff values for immunoglobulin isotopes and BKV infection, sensitivity, specificity, positive predictive value, and negative predictive value were obtained. Power calculations were not performed, as there are no prospectively collected data available on the prevalences of anti-BK IgA, IgM, and IgG antibodies. All statistical analyses were performed using SAS (version 9.1) and Stata (version 8.0) software.

TABLE 1. Serial measurements of anti-BKV antibodies and viral load

Antibody or variable	Median OD (range)					Median log no. of copies/ml (range)				
	1 wk	4 wks	12 wks	6 mos	1 yr	1 wk	4 wks	12 wks	6 mos	1 yr
IgG	0.64 (0.03-2.05)	0.65 (0.02-2.17)	1.04 (0.028-2.24)	1.36 (0.02-2.46)	1.65 (0.02-2.45)					
IgA	0.18 (0.02-1.96)	0.18 (0.016-1.12)	0.29 (0.02-1.76)	0.39 (0.015-1.83)	0.46 (0.019-1.79)					
IgM	0.05 (0.02-0.39)	0.04 (0.01-0.51)	0.06 (0.02-0.79)	0.07 (0.01-0.84)	0.10 (0.02-0.75)					
Urine viral load						0 (0-11.68)	0 (0-11.77)	0 (0-10.97)	0 (0-11.10)	0 (0-9.44)
Plasma viral load						0	0 (0-5.15)	0 (0-5.90)	0 (0-5.31)	0 (0-5.26)

TABLE 2. Baseline serostatus and immunoglobulin optical density in patients classified by virologic outcome

Serostatus or antibody	No. (%) of patients with:			OD for patients with:		
	No BKV	BK viruria	BK viremia	No BKV	BK viruria	BK viremia
Seronegative	5 (11.6)	11 (17.2)	2 (14.3)			
IgG <sup>+</sup> only	12 (27.9)	19 (29.6)	8 (57.1)			
IgG <sup>+</sup> IgM <sup>+</sup>	0	2 (3.1)	0			
IgG <sup>+</sup> IgA <sup>+</sup>	19 (44.2)	27 (42.2)	2 (14.3) <sup>a</sup>			
IgG <sup>+</sup> IgM <sup>+</sup> IgA <sup>+</sup>	7 (16.3)	5 (7.8)	2 (14.3)			
IgG				0.75 ± 0.55	0.77 ± 0.6	0.68 ± 0.57
IgM				0.05 ± 0.03	0.06 ± 0.07	0.05 ± 0.05
IgA				0.25 ± 0.21	0.22 ± 0.21	0.15 ± 0.19 <sup>b</sup>

<sup>a</sup> *P* was 0.04 for comparison with nonviremic patients.<sup>b</sup> *P* was 0.08 for comparison with nonviremic patients.

## RESULTS

An overview of the serial serologic and viral load studies performed on these patients is presented in Table 1, and a discussion of the salient features follows.

**Seroprevalence.** The majority of subjects studied had evidence of past exposure to BKV, as judged by the presence of anti-BK IgG antibodies 1 week after transplantation. The proportions of seronegative subjects varied between 11.6 and 17.2% in different categories of patients (Table 2). Anti-BKV IgA antibodies together with anti-BK IgG were found in week 1 samples obtained from 42.2% of patients with BK viruria and, interestingly, in almost the same proportion (44.2%) of patients who were BKV PCR negative. A significantly lower proportion (14.3%) of IgA- and IgG-positive (IgA<sup>+</sup> IgG<sup>+</sup>) patients at week 1 subsequently became viremic patients (*P* = 0.04). Anti-BKV IgM antibodies were never found without anti-IgA or -IgG in any clinical category. However, the combination of IgM, IgA, and IgG antibodies was found in 16.3% of PCR-negative, 7.8% of viruric, and 14.3% of viremic subjects.

**Primary seroconversion.** Sixteen subjects were BKV seronegative at week 1, of whom 14 seroconverted by 1 year

(14/16; 87.5%) (Table 3). In the first sample showing seroconversion, anti-BK IgG alone was detected in five patients and IgA alone in one patient, and combinations of more than one immunoglobulin were detected in eight patients (three IgG-IgA, two IgG-IgM, and three IgG-IgA-IgM). These 14 primary infections were characterized by absence of viruria (3/14; 21.4%), presence of viruria (11/14; 78.6%; two at time points other than those shown in Table 3), or presence of viremia (2/14; 14.3%; both patients were also viruric).

**Seroreversion.** Of the 91 subjects demonstrated to be BKV seropositive at 1 week posttransplant, 17 (18.7%) showed transient loss of all antibodies during the follow-up period (Table 4). Typically, this occurred at 4 weeks to 6 months posttransplant (mean, 17.4 ± 11.9 weeks). Anti-BK IgG antibodies were lost in all patients, anti-BK IgA antibodies in all but two patients (no. 2 and 4), and anti-BK IgM antibodies in three patients (no. 8, 14, and 17). At the time that seroreversion was detected, 8/17 (47%) patients (no. 3, 4, 11, 12, 13, 15, 16, and 17) were found to be viruric. Four (24%) patients had documented viruria prior to diagnosis of seroreversion and continued to excrete virus in urine afterwards (no. 4, 11, 12, and 14).

TABLE 3. BKV seroconversion in kidney transplant recipients<sup>a</sup>

Patient	Serostatus (viral load <sup>b</sup> of urinary sample)				
	1 wk	4 wks	12 wks	6 mos	12 mos
1	—	G	G	G	G
2	—	—	—	—	—
3	—	—	—	—	—
4	—	—	GAM	GAM	GAM
5	—	A	AM	G	—
6	—	G	GA	—	G
7	—	G	G	G	G
8	— (0)	— (0.00)	GM (7.97)	GAM (0)	GAM (0)
9	— (0)	— (5.42)	— (7.10)	GA (NA)	GAM (5.09)
10	— (0)	G (0)	— (6.52)	GAM (7.88)	GAM (8.71)
11	— (0)	— (0)	— (6.98)	GAM (UTQ)	GAM (0)
12	— (0)	GA (0)	GA (7.51)	— (NA)	GAM (UTQ)
13	— (0)	— (0)	GAM (0)	GAM (0)	GAM (5.49)
14	— (0)	GM (5.07)	G (5.90)	GAM (5.64)	— (UTQ)
15	— (0)	GA (11.01)	GAM (UTQ)	GAM (0)	GAM (6.61)
16	— (0)	G (0)	G (8.87)	GAM (6.20)	GAM (UTQ)

<sup>a</sup> The immunoglobulin isotype(s) detected at each time point is designated — (seronegative), G, A, or M. UTQ, unable to quantitate (these samples contained viral DNA below the linear range of the real-time PCR assay [4,000 copies/ml]); NA, not available.

<sup>b</sup> For patients who developed viruria, urinary viral load in log numbers of copies/ml is indicated.



TABLE 4. BKV seroreversion in kidney transplant recipients<sup>a</sup>

Patient	Serostatus (viral load <sup>b</sup> of urinary sample)				
	1 wk	4 wks	12 wks	6 mos	12 mos
1	G	GA	G	—	G
2	G	G	G	—	—
3	G (0)	GA (0)	— (UTQ)	GA (9.01)	GA (5.92)
4	G (5.74)	— (11.32)	GAM (7.48)	GAM (5.1)	GAM (5.42)
5	GA	G	GA	—	G
6	GA	GA	G	—	G
7	GA	—	GA	GA	GA
8	GA	G	GAM	—	—
9	GA	GA	GA	—	GA
10	GA (0)	— (0)	GA (6)	GA (0)	GA (0)
11	NA (NA)	GA (0)	GA (6.98)	— (7.54)	GAM (UTQ)
12	GA (5.53)	GA (8.53)	— (6.65)	GA (6.99)	GAM (0)
13	GA (0)	GA (0)	— (5.05)	NA (NA)	NA (NA)
14	GA (UTQ)	GA (8.94)	GA (9.54)	GAM (5.87)	— (UTQ)
15	GA (0)	GA (0)	GA (0)	— (7.57)	GAM (5.13)
16	GA (0)	— (5.68)	GAM (6.58)	GAM (UTQ)	GAM (UTQ)
17	GAM (0)	— (5.41)	GAM (5.02)	GAM (0)	GAM (0)

<sup>a</sup> The immunoglobulin isotype(s) detected at each time point is designated — (seronegative), G, A, or M. UTQ, unable to quantitate (these samples contained viral DNA below the linear range of the real-time PCR assay [4,000 copies/ml]); NA, not available.

<sup>b</sup> For patients who developed viruria, urinary viral load in log numbers of copies/ml is indicated.

One patient (no. 10) became viruric for the first time after seroreversion. Seven of seventeen patients (41.0%) did not develop viruria or viremia at any time during the 1-year period of observation (no. 1, 2, and 5 to 9).

**Reactivation infections.** Patients with only anti-BK IgG antibodies detected at week 1 were interpreted as having been exposed to BKV infection prior to transplantation ( $n = 31$ ). The subsequent appearance of anti-BK IgA and anti-BK IgM antibodies was used to indicate a posttransplant reactivation infection. Patients with anti-BK IgA or IgM detectable in the week 1 sample itself were not classified as having reactivation infection, since, in the absence of a day zero sample, the less likely possibility of a primary infection could not be ruled out. In 22/31 (70.9%) patients who could be categorized as having reactivation infection, IgA was detected first in 13 patients and IgM first in 1 patient. In eight patients, IgA and IgM appeared to develop simultaneously, according to the sampling time points chosen for this study (data not shown). Viruria developed in 16/22 (72.7%) and viremia in 7/22 (31.8%) patients, and these proportions are similar to those observed in patients undergoing primary seroconversion (see above).

**Diagnostic value of antibody isotypes.** Immunoglobulin titers as measured by OD were higher for all three classes in samples taken at the time of positive PCR from urine or plasma than in samples taken when PCR showed no evidence of BKV replication (anti-BK IgG,  $1.43 \pm 0.74$  versus  $0.88 \pm 0.64$ ; anti-BK IgA,  $0.59 \pm 0.46$  versus  $0.30 \pm 0.32$ ; anti-BK IgM,  $0.19 \pm 0.19$  versus  $0.08 \pm 0.09$ ) (Table 5). OD cutoff values of 1.8, 0.84, and 0.24 for IgG, IgA, and IgM, respectively, could predict urine PCR-positive and PCR-negative time points with 32.6 to 95.1% specificity and 28.6 to 41.7% sensitivity (Table 6). The positive predictive values and negative predictive values were in the range of 64.6 to 71.7% and 76.0 to 78.5%, respectively. Sensitivity of detection was significantly improved by taking into account temporal changes in antibody titer.  $\Delta$  anti-BK IgG,  $\Delta$  anti-BK IgA, and  $\Delta$  anti-BK IgM cutoff values of 0.38, 0.19, and 0.04, respectively, were

associated with diagnostic sensitivities of 85.9, 88.0, and 76.6%, respectively (Table 6). The negative predictive values were also improved by 10 to 20%, but the positive predictive values were not affected. While the higher ODs in viremic patients could, in part, be due to reduction in immunosuppression, this cannot be said about the viruric patients, in whom a positive PCR test result did not trigger any therapeutic manipulation.

**Temporal relationships between humoral immune response and onset of viral replication.** In patients with primary infection (Table 3), a positive PCR result preceded detection of anti-BK IgA or anti-BK IgM antibodies in four of nine (44.4%) patients who became viruric (no. 9, 10, 11, and 16). Seroconversion and positive PCR results were noted at the same time point in three of nine (33.3%) patients (no. 8, 14, and 15) (Table 1). In two of nine (22.2%) patients (no. 12 and 13), anti-BK IgA or anti-BK IgM detection occurred prior to a detectable viruria. A similar temporal relationship was observed in the setting of reactivation infection (data not shown). However, as previously noted, anti-BK IgA antibodies were frequently present at week 1 even in patients who never went on to develop BK viruria. These patients were not classified as having either primary or reactivation infection but were regarded as indeterminate for infection status. When temporal relationships in all patients taken together were considered, the mean time to anti-BK IgA detection ( $6.9 \pm 11.4$  weeks) was found to be less than the time to detection of viral DNA ( $14.6 \pm 14.9$  weeks) or anti-BK IgM antibodies ( $16.8 \pm 16.7$  weeks).

**Relationships between antibody titers and viral load.** Baseline anti-BK IgA OD at week 1 (Table 2) tended to be lower in patients who went on to develop viremia ( $0.15 \pm 0.19$ ) than in those who developed viruria ( $0.22 \pm 0.21$ ) or no viruria ( $0.25 \pm 0.21$ ) ( $P$  values of 0.08 for viremic versus nonviremic patients). Viremic subjects also had a lower baseline seroprevalence of anti-BK IgA ( $P = 0.04$ ) (Table 2). Baseline anti-BK IgG OD showed a weak inverse correlation with peak urine viral load ( $r = -0.25$ ;  $P = 0.05$ ) (Fig. 1, upper). Direct correlations were observed between urine viral load and  $\Delta$  OD for all three

TABLE 5. Immunoglobulin isotypes in patients with and without BK viruria ( $P = 0.0001$ )

Status (no. of patients)	Mean OD (SD, median, range) for:			$\Delta$ OD <sup>a</sup> (SD, median, range) for:		
	IgA	IgM	IgG	IgA	IgM	IgG
No BKV (364)	0.30 (0.32, 0.21, 0.02 to 1.96)	0.08 (0.09, 0.05, 0.01 to 0.61)	0.88 (0.64, 0.77, 0.02 to 2.33)	0.17 (0.27, 0.09, -0.20 to 1.2 <sup>b</sup> )	0.04 (0.07, 0.02, -0.01 to 0.34)	0.28 (0.42, 0.19, -0.61 to 2.0)
BK viruria (168)	0.59 (0.46, 0.49, 0.02 to 1.76)	0.19 (0.19, 0.09, 0.01 to 0.84)	1.43 (0.74, 1.62, 0.02 to 2.46)	0.67 (0.40, 0.7, 0 to 1.47)	0.23 (0.22, 0.16, -0.07 to 0.80)	1.16 (0.66, 1.17, -0.29 to 2.26)
Total (532)	0.39 (0.40, 0.27, 0.12 to 1.96)	0.11 (0.14, 0.05, 0.01 to 0.84)	1.06 (0.72, 1.06, 0.02 to 2.46)	0.47 (0.43, 0.35, -0.20 to 1.47)	0.16 (0.20, 0.07, -0.07 to 0.80)	0.81 (0.072, 0.62, -0.61 to 2.26)

<sup>a</sup>  $\Delta$  OD is the difference between baseline and peak ODs for each patient.  
<sup>b</sup> A negative number indicates that the baseline was measured at the highest available value.

TABLE 6. Diagnostic utility of measuring immunoglobulin isotypes in patients with BK viruria<sup>a</sup>

Antibody	OD cutoff	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	ROC/AUC
IgA	0.84	31.6	32.6	66.5	78.5	0.68
IgM	0.24	28.6	95.1	71.7	75.7	0.69
IgG	1.8	41.7	91.2	64.6	76.0	0.71
$\Delta$ IgA	0.19	88.0	72.0	57.4	93.3	0.84
$\Delta$ IgM	0.04	76.6	76.1	57.9	98.7	0.82
$\Delta$ IgG	0.38	85.9	71.7	56.6	92.3	0.87

<sup>a</sup> Abbreviations: PPV, positive predictive value; NPV, negative predictive value; ROC/AUC, receiver operating characteristics/area under the curve;  $\Delta$ IgA,  $\Delta$  anti-BK IgA.

immunoglobulin isotypes ( $r = 0.36$  to  $0.43$ ;  $P = 0.004$  to  $0.0004$ ) (Fig. 1, lower). Peak plasma viral load showed no correlation with baseline OD (Fig. 2, upper) but showed direct correlation with  $\Delta$  anti-BK IgM and  $\Delta$  anti-BK IgA (Fig. 2, lower) ( $r = 0.52$  and  $0.37$ ;  $P = 0.001$  and  $0.003$ , respectively).

DISCUSSION

IgG antibodies to BKV VLPs have been measured by several groups of investigators (5, 20, 23). All have reported marked increases in anti-BK IgG antibody titer under conditions associated with viral replication (viruria, viremia, or nephropathy). Elevation of anti-BK IgA and anti-BK IgM class antibodies in the same clinical setting has been reported in a retrospective cross-sectional study of kidney transplant patients at the University of Pittsburgh (42). The current study examined antibody class and OD in a prospectively collected set of samples. Serial antibody (IgG, IgA, and IgM) and PCR measurements provided a direct estimate of the prevalence of viral replication in primary BKV infection. While the seroconversion rate was high, only a subset of patients went on to develop viruria or viremia. Notably, anti-BK IgA antibodies indicated ongoing viral infection in several patients who were BKV PCR negative. This is a novel observation that may be relevant to the control of BKV infection in humans.

In organ transplant recipients, primary infection with Epstein-Barr virus and cytomegalovirus is considered to be more severe than reactivation infection (8, 9, 48, 49, 53). Experience with BKV infection in kidney transplant recipients is conflicting. Bohl et al. noted a 30.8% rate of viruria in BKV-seronegative transplant recipients of a seropositive organ (5). Ginevri et al. initially reported that recipient seronegative status, as assessed by a hemagglutination inhibition assay, conferred a significant risk of viral reactivation (58.3%) in relation to seropositive recipients (21.4%) (21). However, they could not confirm this in a larger follow-up study using ELISA technology (20). Hirsch et al. noted that 78.2% of patients shedding virus-replicating “decoy cells” were seropositive at the time of transplantation, while 21.8% were seronegative (26, 50). Smith et al. found that 83.3% of pediatric patients with BKV nephropathy were seronegative for anti-BK IgG antibodies (50). These discrepancies likely reflect patient selection, small sample size, geographical differences in viral epidemiology, and possibly differences in technical methods of assaying anti-BKV antibodies. It is also possible that the effect of serostatus at the time of infection can be overridden by other, more-important



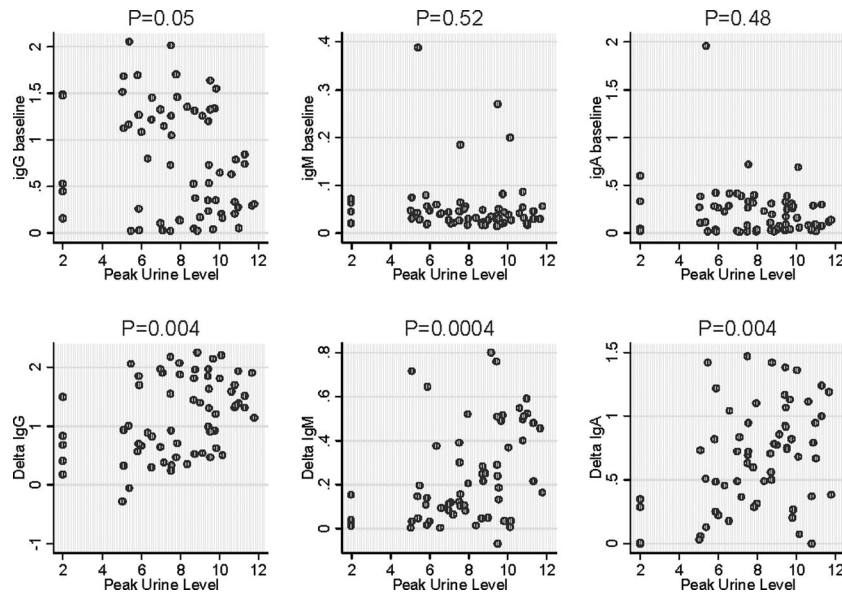


FIG. 1. Correlation between immunoglobulin measurements and peak viral load in urine. Baseline OD (upper) showed a weak inverse correlation for anti-BK IgG, IgM, and IgA, but only the relationship with IgG OD was statistically significant ( $r = -0.25$ ;  $P = 0.05$ ). When  $\Delta$  OD was substituted for baseline OD (lower panel), direct correlations with viral load were found for all three immunoglobulin isotypes ( $r = 0.36$ ,  $0.36$ , and  $0.43$  for  $\Delta$  anti-BK IgG [Delta IgG],  $\Delta$  anti-BK IgA, and  $\Delta$  anti-BK IgM, respectively).

factors, such as strength of the cell-mediated immune response, overall state of immunosuppression, and host or viral gene polymorphisms.

Seroreversion has not previously been recorded in the setting of BKV infection. Neither viruria nor viremia was detectable in seven patients showing this phenomenon, and it could be argued that seroreversion reflected a loss of antigenic stimulation. Several patients had viruria present

from a time preceding or concurrent with diagnosis of seroreversion. In the latter group of cases, it is possible that the loss of ability to sustain antibody production reflects loss of BKV-specific memory B cells (24). The underlying cause is unclear as there was no augmentation of immunosuppression in these individuals; indeed, immunosuppression was reduced in the patients who became viremic. Antiviral treatment has been reported to result in seroreversion in human

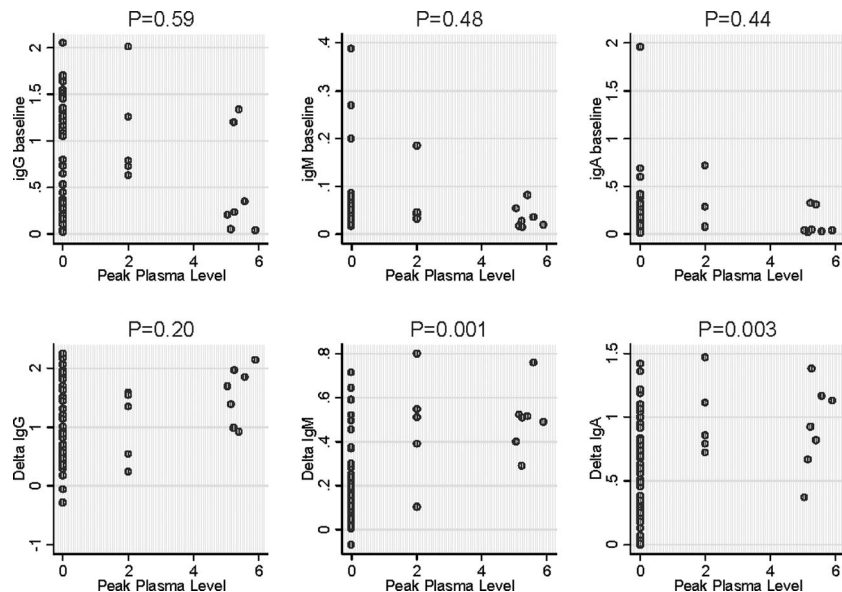


FIG. 2. Correlation between immunoglobulin measurements and peak viral load in plasma. Baseline OD (upper) did not show any relationship with peak plasma viral load. When  $\Delta$  OD was substituted for baseline OD (lower panel), a direct correlation was observed with  $\Delta$  anti-BK IgM [Delta IgM] and  $\Delta$  anti-BK IgA ( $r = 0.52$  and  $0.37$ ;  $P = 0.001$  and  $0.003$ , respectively). Note that the large majority of samples tested negative for plasma BKV DNA.

immunodeficiency virus or hepatitis C virus infection, but none of our patients received cidofovir, leflunomide, or intravenous Ig (2, 29, 32). It is likely that in some patients, the immunosuppressed state can itself result in a dysregulated antibody response (3, 25, 33, 38, 46).

With respect to the implications of our findings for clinical diagnosis, assaying anti-BK IgA and anti-BK IgM antibodies could certainly provide one means for detecting viral activation in transplant recipients. Evaluation of at least two samples would be needed to calculate  $\Delta$  OD, since single-point measurements were less reliable in distinguishing PCR-positive and PCR-negative samples. However, current clinical paradigms of therapeutic intervention in BKV infection are guided by the presence of active viral replication, as evidenced by persistent high-level viruria ( $>1E+07$  copies/ml), viremia, or nephropathy. Quantitative PCR is, therefore, more suited as a primary screening test for BKV in kidney transplant patients, even though it cannot detect past exposure, exposure at sites not related to the body fluid tested, or a recent episode of reactivation with subsequent viral clearance. On the other hand, antibody measurements could potentially provide information of prognostic value. In our data set, baseline anti-BK IgG OD showed an inverse correlation with peak urine viral load. Patients who went on to develop viremia had a lower baseline seroprevalence of anti-BK IgA. These observations raise the possibility that preexisting antibody titers play a protective role and modulate the ultimate clinical outcome of posttransplant BKV infection. Such a preemptive protective role is not inconsistent with the opinion of some investigators that antibodies are not very effective in controlling active viral replication in patients with active BKV nephropathy (4, 5). It is likely that anti-VLP antibodies act primarily by binding viral capsids to prevent intracellular BKV entry and work efficaciously only in initial stages of infection. Analogous findings have been reported by Khoury et al., who studied cytomegalovirus infection and observed that donor-seronegative, recipient-seropositive kidney transplant patients show less-severe viremia than donor-seropositive, recipient-seronegative patients (30). However, such considerations may not apply to BKV infection in bone marrow transplant recipients, who typically undergo intense immunosuppression to maximize the chance of donor marrow engraftment (7, 52).

In summary, serologic evidence of primary and reactivation BKV infection is common in kidney transplant patients, but not all patients develop active viral replication. Primary and reactivation infections carry similar risks of viruria and viremia, perhaps pointing to the importance of a donor-derived infection in both instances. Finally, although larger confirmatory studies are needed, it appears that immunoglobulin class measurement might provide some prognostic information: in this series, low anti-BK IgG and IgA titers were associated with higher peak urinary viral loads and greater risk of viremia.

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